

“Self-Absorption” Phenomenon in Near-Infrared Fourier Transform Raman Spectroscopy of Cellulosic and Lignocellulosic Materials

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INTRODUCTION

While cellulosic and lignocellulosic materials have been studied using conventional Raman spectroscopy, availability of near-infrared (NIR) Fourier transform (FT) Raman instrumentation has made studying these materials much more convenient.¹ This is especially true because the problem of laser-induced fluorescence can be avoided or minimized in FT-Raman (NIR Raman) spectroscopy. More recently, the method has also been used to generate quantitative results in cellulosic and lignocellulosic materials.²⁻⁵

Although linear dependence of Raman intensity on analyte concentration is well established, the occurrence of self-absorption (defined as absorption of Raman scattered photons by the sample itself) is known to cause problems.^{6,7} The influence of self-absorption in an FT-Raman spectrum is manifested in the spectral region where the sample absorbs as a result of overtone- and combination-vibration bands. The net result is that the Raman band intensity or intensities are diminished. This is also true for samples that have an electronic absorption in the visible region and are being analyzed by visible Raman spectroscopy. For instance, researchers have pointed out the occurrence of self-absorption in resonance Raman spectroscopy.^{8,9} Compared to visible Raman spectroscopy, where only a small number of samples have strong electronic absorption, in NIR Raman, many more substances are expected to have overtone and combination-

vibration transitions. Additionally, in the latter method, because sampling is deeper, self-absorption can be more problematic.

For the present investigation, cellulose filter paper and spruce thermomechanical pulp (TMP) were chosen as representatives of cellulose and lignocellulose, respectively. The objective was to determine whether self-absorption was present in the spectra and which Raman bands were suppressed as a consequence.

METHODS AND MATERIALS

Cellulose Whatman 1 qualitative filter papers were purchased from Cole-Parmer Instrument Company (Ver-non Hills, IL). Spruce TMP was a gift from Consolidated Papers (now Stora Enso North America, Wisconsin Rapids, WI). Milled wood lignin (MWL) was isolated from black spruce wood using the published isolation procedure.¹⁰ TMP pellets were made using an IR pellet press with the hydraulic pressure kept below 2812 tons/m².

The FT-Raman instrument was Bruker's RFS-100 (Bruker Optics, Billerica, MA) equipped with an Nd:YAG laser capable of producing 1 W of polarized laser power output at 1064 nm. Samples were excited by 300 mW of laser power, and 600 scans were accumulated with the use of a double-sided forward-backward scanning mode. Samples were analyzed in the 180° scattering geometry, and a sample stage micrometer was used to position the sample along the optical axis of the spectrometer. Spectra were obtained from several sample positions around the focal plane. In some measurements, a front-surface coated mirror was positioned behind the sample to determine how band intensities within a spectrum changed (relative to other bands). Sample thickness and moisture changes were also investigated. Samples were thin (0.18-mm) strips of filter paper and pressed pellets of TMP (1 and 3 mm) and lignin (2 mm). NIR measurements were carried out on a Bruker's IFS66 equipped with a Spectra-Tech diffuse reflectance accessory (Thermo Electron, Lanham, MD).

For the purpose of comparing intensities, spectra were normalized using the 380 cm⁻¹ band of cellulose. This band was chosen because its intensity is not expected to be influenced by self-absorption since cellulose¹¹ and water⁶ do not have any absorption at the corresponding wavenumber in the NIR (9018.5 cm⁻¹). When spectra were normalized using the 380 cm⁻¹ band, all peaks, with the exception of the 2895 cm⁻¹ peak, were found to match in intensity. This implied that bands other than 380 cm⁻¹ could be used for normalization purposes. Considering that the 1096 cm⁻¹ band is stronger than the 380 cm⁻¹ band, the former was chosen to calculate relative peak intensities. The sloping background method was used for peak height band intensity calculations. Repeat measurements under identical conditions (same relative

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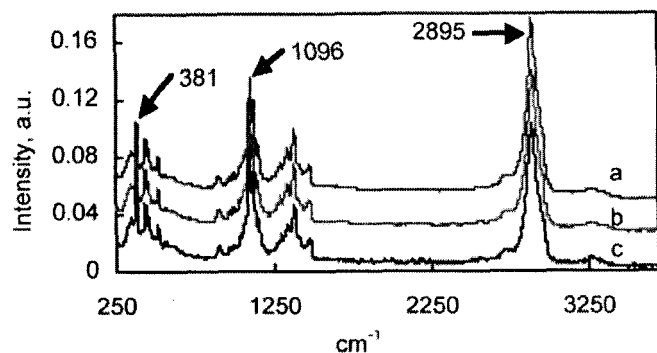


FIG. 1. Effect of sample position on band intensity for cellulose Whatman 1 filter paper: (a) in focus, (b) 1.5 mm away from focus, and (c) 3 mm away from focus. Spectra were normalized on the 381 cm^{-1} band. Only the 2895 cm^{-1} band declined in intensity. For clarity, only three of the six obtained spectra are shown.

humidity) indicated that the relative peak height data for the 2895 cm^{-1} band differed by only 2.3%.

RESULTS AND DISCUSSION

Effect of Sample Position. When self-absorption is present, band intensity in a Raman spectrum depends upon the sample position.¹² To detect self-absorption, samples of cellulose (filter paper) and lignocellulose (TMP) were studied at various sampling positions. The spectra shown in Fig. 1 were normalized on the 380 cm^{-1} band of cellulose present in the spectra of the filter paper. Similar spectra (not shown here) were obtained for the TMP.

When the relative intensities of bands were calculated with respect to the 1096 cm^{-1} Raman band, it was found that except for the 2895 cm^{-1} band, the intensities of all other bands were similar between spectra. This indicated that the C–H stretch band intensity was suppressed as a result of self-absorption. In the spectrum of spruce wood, the 2895 cm^{-1} band has been assigned to cellulose;¹⁰ the same assignment is expected for TMP because all the components are retained when the pulp is produced. For both the filter paper and TMP, the 2895 cm^{-1} band intensity declined to varying degrees depending upon the position of the sample on the optical axis. The effect of sampling position on band intensity is shown in Fig. 2. For calculating the relative band intensities plotted in Fig. 2, the reference Raman band at 1096 cm^{-1} was chosen because this band is moderately intense and occurs at 8302 cm^{-1} (absolute frequency), where no significant absorption is detected in the NIR spectra of cellulose, spruce milled wood lignin (MWL), and TMP NIR spectra of these materials are shown in Fig. 3. MWL is the closest representation of TMP lignin, and the isolation procedure does not cause any significant change in molecular structure of the lignin (compared to its structure in pulp or wood).

The similarity of behavior of the 2895 cm^{-1} band for the two samples (Fig. 2) is not surprising given that in TMP the band is largely due to cellulose. The intensity of the 2895 cm^{-1} band becomes more suppressed as a sample moves towards the spectrometer because the instrument collects Raman scattered photons from the deeper parts of the sample and, therefore, the Raman pho-

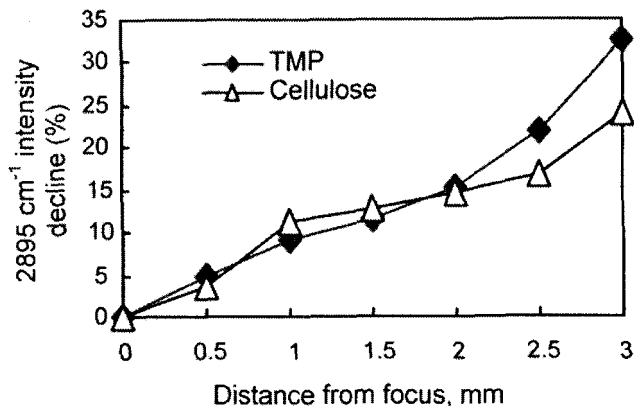


FIG. 2. Decline in intensity of the 2895 cm^{-1} band in FT-Raman spectra of filter paper and TMP. Decline was higher for sampling positions further from the focal plane.

tons have to travel a longer distance inside the sample. This deeper/longer aspect means more opportunities for a Raman photon to be absorbed by the sample and thus get lost before reaching the detector.

Because the 1600 cm^{-1} band¹⁰ of lignin showed similar peak intensity in the normalized TMP spectra (not shown here) obtained from different positions, the implication is that self-absorption is not an important consideration for quantitative analysis of lignin.

Effects of Sample Thickness and Mirror Polishing.

The effect of sample thickness on self-absorption was evaluated by comparing 1- and 3-mm-thick TMP pellets and comparing a single 0.18-mm-thick strip of filter paper to a sample of three 0.18-mm-thick strips. For a given sample position, the thicker the sample the greater the decline in the relative peak intensity of the band at 2895 cm^{-1} (Fig. 4 and Table I). TMP showed a slightly greater decline compared to that of the filter paper. This difference was likely due to the fact that the TMP pellets were much thicker than the filter paper strips. For both materials, these results can be understood in terms of higher absorption of the Raman scattered photons in a thicker sample.

To increase signal in FT-Raman, a front-surface coated mirror is often put behind thin samples. Based on our

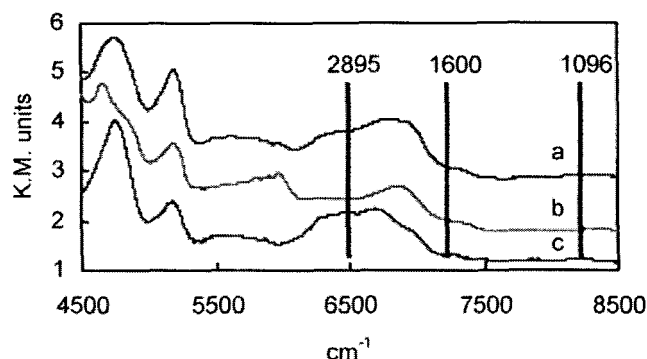


FIG. 3. NIR diffuse reflectance spectra of (a) TMP, (b) MWL, and (c) filter paper cellulose. For display purposes, spectra (a), (b), and (c) were y-offset by 2.50, 1.50, and 1.00 K.M. units, respectively. For all materials, no absorption observed at 8302 cm^{-1} (corresponding to Raman shift of 1096 cm^{-1}); for TMP and filter paper, significant absorption observed at 6503.5 cm^{-1} (corresponding to 2895 cm^{-1} Raman shift). Vertical lines indicate location of various Raman bands.

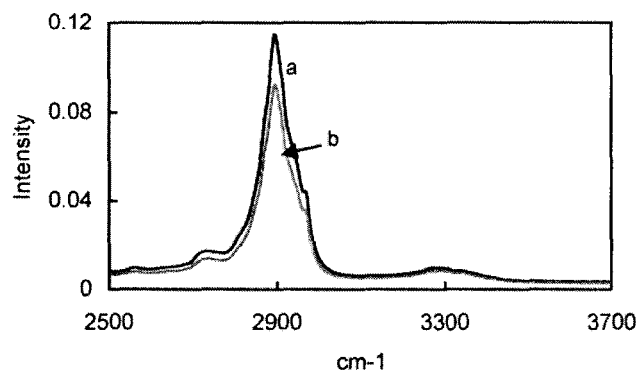


FIG. 4. Effect of filter paper thickness on suppression of 2895 cm^{-1} band: (a) single 0.18-mm strip and (b) sample of three 0.18-mm strips. Spectra were normalized on the 1096 cm^{-1} band (not shown).

analysis of the spectra of thin and thick samples of filter paper and TMP, the use of a mirror suppressed the 2895 cm^{-1} band only in the thin samples (Fig. 5 and Table I). Results from using a mirror with thick samples are not reported here because no intensity decline was observed. The suppression of intensity was found to be significantly smaller for TMP (Table I), possibly because the TMP pellet was much thicker than the filter paper sample, and, as noted above, the mirror effect diminishes with an increase in sample thickness. For thin samples, using a mirror is equivalent to increasing the sample thickness because the laser beam makes an additional pass, after being reflected from the mirror, from the back to the front of the sample. For thick samples, on the other hand, the beam is already diffused and weak, and reflection from the mirror is therefore negligible.

Role of Water. In NIR, water absorbs strongly in the 9500–5500 cm^{-1} range.⁶ Comparison of the NIR absorption spectra of water, cellulose, and TMP led to the conclusion that the hydroxyl groups in the samples are in large part responsible for the suppression of the FT-Raman bands in the C–H stretch region. To further evaluate the role of water and carbohydrate O–H groups, filter paper Raman spectra were obtained both before and after adding a small amount (25 μL) of water (Fig. 6). As expected, after adding water, the intensity of the 2895 cm^{-1} band declined substantially (Fig. 6b, Table I) but it recovered as the water evaporated (Fig. 6c, Table I). Water evaporation was evidenced by the Raman intensity decline in the O–H stretch region (Fig. 6). The addition of water clearly diminished the intensity of the 2895 cm^{-1} C–H stretch band in the filter paper sample. No spectra

TABLE 1. Peak height of 2895 cm^{-1} band, after normalizing on 1096 cm^{-1} band.

Sample	I_{2895} decline (%)	
	Filter paper	Spruce TMP
Thick sample ^a	18	21.6
Thin sample with mirror ^b	9.9	4.5
Control plus 25 μL H ₂ O	31.5	NA ^c
Control after water evaporation	9.9	NA

^a Whatman 1 filter paper, three 0.18-mm-thick strips; TMP, 3-mm-thick pellet.

^b Whatman 1 filter paper, single 0.18-mm-thick strip; TMP, 1-mm-thick pellet.

^c Not available; see text.

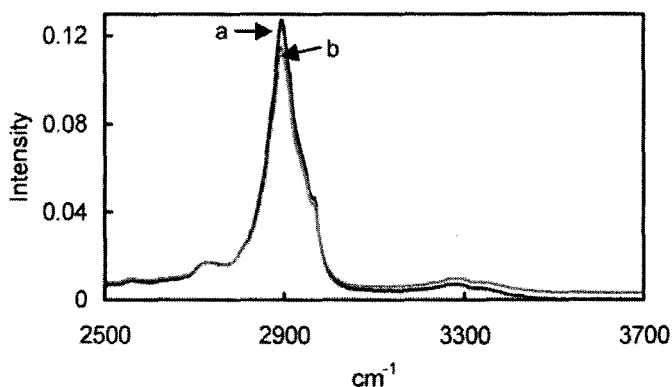


FIG. 5. Role of mirror in suppression of 2895 cm^{-1} band for filter paper: (a) without mirror and (b) with mirror. Spectra were normalized on the 1096 cm^{-1} band (not shown).

were obtained for the TMP because the addition of water caused significant swelling and pulp fibers detached from the surface of the pellet. This was due to weaker inter-fiber interactions in TMP compared to filter paper.

Implications for Quantitative Work. In FT-Raman analysis, one simple way to determine whether or not self-absorption is likely to be a problem is to obtain a NIR spectrum of the sample (or from the literature if available) and determine if, over the range of Raman measurement, any NIR absorption is present. In case NIR absorption is present and self-absorption is predicted in FT-Raman, thin samples ought to be used wherever possible because they are less prone to band suppression. Using samples with identical thickness may also be warranted, so that an effect will not vary from one sample to the next. Lastly, in quantitative work, self-absorption problems could be avoided by avoiding peaks in the C–H stretch region. If an FT-Raman investigation calls for using water, replacing H₂O with D₂O should be considered because the NIR spectra of these compounds are quite different.⁶

CONCLUSION

The self-absorption effect was found to be present in cellulosic filter paper and spruce thermomechanical pulp samples. The effect suppressed the intensity of the 2895 cm^{-1} band in the C–H stretch region of the FT-Raman spectra. Experimental observations indicated that such

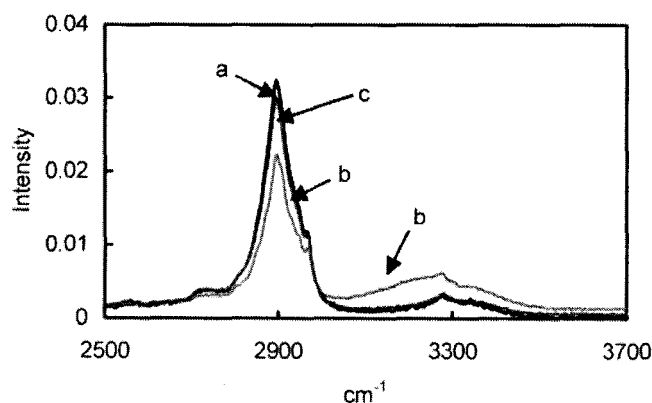


FIG. 6. Effect of added water on filter paper spectrum: (a) control, (b) with addition of 25 μL H₂O, and (c) after most water had evaporated.

suppression was caused by cellulose in both samples and lignin was not involved. In TMP and cellulosic filter paper, suppression was caused by the hydroxyl groups, both in carbohydrates and water. Although certain precautions can be taken in quantitative work to minimize the detrimental effect of this phenomenon, it is best to choose a band that is not in the C-H stretch region of the spectrum.

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